Polymorphism in the promoter region of the mannose-binding lectin gene among human T-cell lymphotropic virus infected subjects

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The present study investigated the frequency of the mutations at positions -550 and -221 of the mannose-binding lectin (MBL) gene in a sample of 75 human T-cell lymphotropic virus (HTLV) infected patients and 96 HTLV seronegative controls, in order to evaluate the occurrence of a possible association between the polymorphism and HTLV infection. A sequence specific primer-polymerase chain reaction was used for discrimination of the polymorphism. The analysis of allele frequencies at position -550 did not show any significant differences between HTLV infected group and controls, but there was a significant difference at position -221. The comparative analysis of haplotypes frequencies were not significant, but the genotype frequencies between the two groups, revealed a higher prevalence of genotype LYLX (25.3%), associated with medium and low MBL serum levels among HTLV infected subjects. The odds ratio estimation demonstrated that the presence of genotype LYLX was associated with an increased risk of HTLV infection ($p = 0.0096$; $1.38 \leq IC95\% \leq 7.7605$). There was no association between proviral load and the promoter polymorphism, but when promoter and exon 1 mutations were matched, it was possible to identify a significant higher proviral load among HTLV infected individuals carrying haplotypes correlated to low serum levels of MBL. The present study shows that the polymorphism in the promoter region of the MBL gene may be a genetic marker associated with HTLV infection, and emphasizes the need for further studies to determinate if the present polymorphism have any impact on diseases linked to HTLV infection.

Key words: mannose-binding lectin - genetic polymorphism - human T-cell lymphotropic virus

Mannose-binding lectin (MBL) is a liver-derived serum lectin with an important role in the host's innate immune system, which binds with high affinity to mannose or other carbohydrate components present on the surface of viruses, bacteria and yeasts (Turner 2003). The MBL is an acute-phase protein witch may mediate phagocytosis or activate the lectin pathway of complement (Kilpatrick 2002).

The identification of the mutation in the promoter region of the MBL gene showed that serum MBL concentration is also modulated in transcriptional levels (Madsen et al. 1995). Nucleotide substitutions in positions -550 (G to C) and -221 (G to C) provide the variants H(G)/L(C) and Y(G)/X(C), respectively. Several promoter variants which apparently do not alter the levels of MBL were described, but HY, LY and LX haplotypes are currently associated to high, medium and low levels of circulating MBL in the plasma, respectively (Madsen et al. 1995, Steffensen et al. 2000). MBL function is directly associated with its serum concentration which is determined by the interplay between promoter and structural gene mutations (Madsen et al. 1995, Jüliger et al. 2000).

Human T-cell lymphotropic virus 1 and 2 (HTLV-1 and HTLV-2) are members of the family *Retroviridae* and share several molecular and biological properties (Hall et al. 1994). HTLV-1 is endemic in diverse geographical regions and is associated with adult T-cell leukemia/lymphoma (ATLL) and a neurodegenerative disorder named tropical spastic paraparesis/myelophaty associated to HTLV-1 (TSP/HAM) (Gessain et al. 1985, Osame et al. 1986). The vast majority of HTLV-1-infected individuals remain asymptomatic, suggesting that HTLV-1 infection alone is not sufficient to cause HTLV-1-related diseases. Furthermore, until now it is unclear why certain individuals develop ATLL and others develop TSP/HAM, but genetic factors seem to be important (Nishimura et al. 2000, 2003, Nitta et al. 2003)

Recently, we have shown a strong association between structural MBL gene mutations and the susceptibility and proviral load of HTLV-1 (Pontes et al. 2005). The present study investigated the association between polymorphism in the promoter region of the mannose-binding lectin gene and the susceptibility to HTLV infection.

SUBJECTS, MATERIALS AND METHODS

Subjects - A total of 75 blood samples from HTLV infected asymptomatic subjects (51 HTLV-1 and 24 HTLV-2; 46 males and 20 females, mean age 40 years), attended in the Blood Bank of the state of Pará (PA), Brazil (HEMOPA), which were enrolled in our previous studies concerned about exon 1 polymorphisms in the MBL gene (Pontes et al. 2005). Additionally, 99 HTLV seronegative subjects residing in Belém, capital of PA, were included in the present study as control group.

Financial support: CNPq, Funtec/Sectam, Universidade Federal do Pará
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Recieved 23 August 2007
Accepted 18 December 2007

Blood samples, from both HTLV infected patients and controls, were collected in Vacuntainer tubes containing K_3-EDTA as anticoagulant in order to obtain plasma and peripheral blood mononuclear cells (PBMC) and screened for HTLV-1/2 antibodies using an enzyme-linked immunosorbent assay (Ortho Diagnostic, Raritan, NJ, USA). Blood samples were directed to the Virus Laboratory (Institute of Biological Sciences, Federal University of Pará) to investigate the MBL gene polymorphisms. All subjects signed a thoroughly-explained consent form at the first visit.

MBL genotyping - Genomic DNA was extracted from PBMC using the purification kit GFX for genomic DNA (Amersham Pharmacia Biotech, USA). Sequence specific primer-polymerase chain reaction (PCR) was used for discrimination of the polymorphism at -550 (316pb [H-550]/[L-550]) and -221 (440pb [X-221] and 443pb [Y-221]) positions of the MBL gene promoter using a Mastercycler Personal termocycler (Eppendorf) following the protocol as previous reported (Steffensen et al. 2000).

Proviral load quantification - The quantification of HTLV proviral load was performed by a real-time PCR assay, as previous described (Tamegão-Lopes et al. 2006).

Statistical analysis - The statistical significance of the differences found between the allele and genotype frequencies were estimated by the χ^2 test followed by Yates' correction. The association analysis between genetic polymorphism and the mean values of proviral load count was performed by the ANOVA test, followed by Bonferroni'correction. Both tests were performed using the software BioEstat 4.0 (Ayres et al. 2005).

RESULTS

The study showed in the -550 position the presence of alleles *MBL*H* and *MBL*L* with frequencies of 38% and 62% among HTLV-infected subjects and 38.5% and 61.5% among healthy controls, respectively. At the position -221, the alleles *MBL*X* and *MBL*Y* showed frequencies of 26% and 64% among HTLV-infected subjects and 16.1% and 83.9% among healthy controls, respectively. The differences observed in the allele frequencies were not statistically significant for -550 position (p = 0.992; Table I), but the observed frequencies at the -221 region revealed a significant difference between the two groups (p = 0.035; Table I).

The genotype differences between the two groups were statistically significant in the -221 position (χ^2 = 13.534; p = 0.0012; Table I). The highest level of significance was found in the frequencies of the genotypes YY and YX, which were 48% and 52% among HTLV-infected and 71% and 26% among controls.

The odds ratio estimation (OR) showed that the presence of X allele (OR = 1.825; p = 0.035; 1.074 ≤ IC95% ≤ 3.100) was associated with an increased risk for HTLV infection. On the other hand, a low risk for HTLV infection was evidenced for those patients carrying the Y allele (OR = 0.548; p = 0.035; 0.322 ≤ IC95% ≤ 0.931).

The differences in the observed haplotype frequencies were not statistically significant (χ^2 = 6.179; p = 0.103; Table II), but the genotype frequencies between

TABLE I

Distribution of allele and genotype frequencies among HTLV infected subjects and controls

Polymorphism	HTLV infected n (%)	Controls n (%)	x^2	p
Region -550				
H	57 (38)	74 (38.5)	-	-
L	93 (62)	118 (61.5)	-	-
Total	150 (100)	192 (100)	0.000	0.9922
HH	14 (19)	14 (15)	-	-
HL	29 (39)	46 (48)	-	-
LL	32 (42)	36 (37)	1.533	0.4647
Region -221				
X	39 (26)	31 (16.1)	-	-
Y	111 (64)	161 (83.9)	-	-
Total	150 (100)	192 (100)	4.436	0.0352
YY	36 (48)	68 (71)	-	-
YX	39 (52)	25 (26)	-	-
XX	0 (0)	03 (03)	13.534	0.0012

n = chromosomes sampled.

TABLE II

Distribution of haplotype frequencies among HTLV infected subjects and controls

Haplotypes	HTLV infected n (%)	Controls n (%)	x^2	p
HY	53 (35.33)	69 (35.94)	-	-
LY	58 (386)	92 (47.92)	-	-
LX	35 (23.33)	26 (13.54)	-	-
HX	04 (2.66)	05 (2.60)	-	-
Total	150 (100)	192 (100)	6.179	0.103

n = chromosomes sampled.

TABLE III

Distribution of genotype frequencies among HTLV infected subjects and controls

Genotype	HTLV infected n (%)	Controls n (%)	x^2	p
HYHY	10 (13.33)	13 (13.54)	-	-
HYLY	13 (17.33)	30 (31.25)	-	-
HYLX	16 (21.33)	12 (12.50)	-	-
HYHX	04 (5.33)	01 (1.04)	-	-
LYLY	13 (17.33)	25 (26.04)	-	-
LYLX	19 (25.33)	09 (9.38)	-	-
LYHX	0 (0)	03 (3.12)	-	-
LXLX	0 (0)	02 (2.09)	-	-
LXHX	0 (0)	01 (1.04)	-	-
Total	75 (100)	96 (100)	20.214	0.0096

n = number of investigated subjects.

the two groups, revealed a higher prevalence of genotype LYLX (25.3%), which has been associated with medium and low MBL serum levels, among HTLV-infected subjects ($\chi^2 = 20.214$; $p = 0.0096$; Table III). The odds ratio estimation demonstrated that the presence of genotype LYLX was associated with an increased risk of HTLV infection ($p = 0.0096$; $1.38 \leq IC95\% \leq 7.7605$). Additionally, when HTLV-1 and HTLV-2 infection were taken into account, no significant ($\chi^2 = 6.259$; $p = 0.282$) difference of the genotype frequencies was observed.

The quantification of HTLV proviral load according to the presence of genotypes showed no association between the polymorphism in the promoter region with the levels of DNA proviral copies per mm^3 ($p > 0.05$; Table IV). However, when exon 1 polymorphism data, previously published (Pontes et al. 2005), were matched with those on the promoter region described herein (Table V), it was possible to identify a significant higher proviral load between HTLV infected individuals carrying haplotypes correlated to the low serum levels of MBL and those carrying haplotypes associated to high/medium MBL serum levels.

DISCUSSION

MBL is an important serum protein of the host's innate defense against microorganisms due to its ability to activate the complement system during the acute-phase of infection (Kilpatrick 2002), but several studies have shown that the presence of genetic polymorphisms of the promoter region of MBL gene, specially at the positions -550 and -221, affect the serum protein levels (Madsen et al. 1995, Steffensen et al. 2000, Lee et al. 2005) and, consequently, increase the susceptibility to infectious diseases (Madsen et al. 1995, Prohászka et al. 1997).

In the present study the allele and the haplotype frequencies in the promoter region of the MBL gene was described among HTLV-infected subjects and seronegative controls in order to investigate the possible role of the promoter polymorphism in face of an HTLV infection.

At the -550 region there were no significant differences in the allele and haplotype frequencies in both HTLV-infected and controls. On the other hand, at the -221 region it was observed a significant difference due to the higher prevalence of the allele X and the YX haplotype among HTLV-infected. Additionally, there was an increased risk for HTLV infection in subjects carrying the allele X and the genotype LYLX. Regarding that previous studies which have reported that the two promoter polymorphism of -550 (H/L) and -221 (X/Y) are associated with low serum MBL levels and that the effect of -221 promoter polymorphism is stronger than -550 promoter polymorphism (Madsen et al. 1995, Steffensen et al. 2000) we could suggest that -550 promoter polymorphism has no influence on HTLV infection, but the -221 promoter polymorphism seems to be associated with HTLV infection.

The carbohydrate components existent in HTLV can be bond by the MBL oligomeric structure. Thus, it is possible that the polymorphism in the MBL gene, specially that present in the promoter region, can be associated with HTLV infection. As previously suggested the MBL can bind to the virus or infected cells followed by the activation of the complement systems (Garred et al. 2003). Thus the low MBL plasma concentration, and subsequent reduction of the complement activation, could contribute to an increased susceptibility to HTLV infection. Recently, we have demonstrated a high association between coding variant at codon 54 of MBL gene with the susceptibility to HTLV infection (Pontes et al. 2005).

When HTLV-1 and HTLV-2 infections were considered separately, no significant difference of the genotype frequencies was observed between the groups. It is in disaccording with our previous study that identified significant differences for exon 1 polymorphism, even regarding that the previous result could be attributed to the number of investigated HTLV-1-infected subjects being higher than that of HTLV-2-infected individuals (Pontes et al. 2005).

TABLE IV

Quantification of the proviral load according to the MBL gene polymorphism

Genotypes	n	Proviral load[a] (copies/mm^3)	Log_{10}
HYHY	10	178.50	2.251
HYLY	13	282.54	2.451
HYLX	16	212.37	2.327
HYHX	3	64.67	1.810
LYLY	12	192.83	2.285
LYLX	19	202.10	2.305

n = number of investigated subjects. *a*: proviral load and log values in arithmetic mean (p-values > 0.05).

TABLE V

Quantification of the proviral load according to the MBL genotypes associated with MBL serum levels

Genotypes	MBL Level[a]	n	Proviral load[b] (copies/mm^3)	Log_{10}	p
HYA/A, HY/LYA, LYA/A, LY/LXA, HYO/O, HYA/LYO, HYA/LXO	High and Medium	50	143.9	2.15	
LYA/O, LYO/O, LYO/LXA, HY/LXO, LY/LXO	Low	23	357.9	2.55	< 0.05

n = number of investigated subjects. *a*: Garred et al (2003); *b*: proviral load and log values in arithmetic mean.

The differences observed in the allele and haplotype frequencies obtained in the present study and those reported for Caucasians, Japaneses, Africans, Koreans and Eskimos (Madsen et al. 1995, Matsushita et al. 1998, Steffensen et al. 2000, Lee et al. 2005) could be attributed to the differences of genetic background among ethnic groups. The genetic contribution of the populations inhabiting the Brazilian Amazon region indicates a clear trihybrid model in which the contribution of Whites, Indians and Blacks has been estimated about 47, 41 and 12%, respectively (Santos & Guerreiro 1995).

Nishimura et al. (2003) showed the association between the presence of allele MBL*B and low levels of HTLV proviral load, but our previous results suggest that the presence of the coding variant at codon 54 might contribute to an inefficient elimination of the virus and a consequent increase of proviral load (Pontes et al. 2005). We were not able to identify, in the present study, any association between -221 and -550 promoter polymorphisms with the high or low values of proviral load which suggest absence of influence of the promoter polymorphisms. But when matching the polymorphisms results of the promoter and exon 1 regions, identified a significant association between high proviral load and the presence of genotypes correlated to low MBL serum levels, highlighting our previous report showing the direct influence of the structural mutation on exon 1 regarding HTLV proviral load. Thus, the results obtained suggest that the low serum levels of MBL could contribute to an inefficient elimination of the virus and a consequent increase of proviral load.

The results of the present study suggest an association between promoter polymorphism of the MBL gene and HTLV infection. Additionally, it is also important to pursue the possibility of whether the MBL gene polymorphism could have any impact on the diseases associated with HTLV infection.

ACKOWLEDGEMENTS

To all subjects involved in this study.

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